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COCKROACHES AS VECTORS OF BACTERIA IN HOSPITAL ENVIRONMENTS.

By

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APPROVAL FORM

This is to certify that that the dissertation entitled “An assessment of the bacterial load and antibiotic susceptibility patterns of bacteria found in cockroaches obtained at a hospital in Gweru , submitted in partial fulfilment of the requirements for Bachelor of Science Honours Degree in Applied Biological Sciences and Biotechnology at Midlands State University, is a record of the original research carried out by Rumbidzayi Makumana (R146419V)under my supervision and no part of the dissertation has been submitted for any other degree or diploma.

The assistance and the help received during the course of this research have been duly acknowledged. Therefore, I recommend that I will be accepted as fulfilling the dissertation requirements.

Name of supervisor …….

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ABSTRACT

Cockroaches have become one of the most common pests due to their wide distribution in human dwellings, kitchens, food outlets and the hospital. In hospitals their wide abundancy may be related to poor sanitary conditions, plenty of food sources, environments which are unhygienic and moist shady places. This study was carried out to evaluate the microbial fauna of cockroaches in a hospital environment as proxy to Health acquired infections. Two different species of cockroaches *Periplaneta americana* and *Blatella germanica* were collected in the canteen and the main kitchen of a hospital in Gweru. The former and the latter were cultured using standard procedures and antibiotic susceptibility tests were done. Microbial isolation was done for the two species of cockroaches by growing saline suspensions of the cockroach samples onto selective and differential media. Bacterial isolates were identified by biochemical tests. Medically important microorganisms were isolated from their external surface and their internal surface. These bacteria were *Proteus spp, Klebsiella spp, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus.* The susceptibilities of *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella spp, Escherichia coli, and Proteus* species to 6 antibiotics were tested. Most of the gram negative bacteria were resistant to amoxicillin, Augmentin and streptomycin and sensitive to ciprofloxacin and chloramphenicol. Enumeration was also conducted using saline suspensions of the internal and external washings and the range from *P. americana* to *B. germanica* from external washings were 3.60×10^4 to 3.20×10^4 cfu/ml respectively and for the internal washings 3.40×10^4 to 3.23×10^4 cfu/ml respectively .The data collected was subjected to Two-way analysis of variance (ANOVA) using SPSS version 21. Two-way Anova results showed that there is no statistically significant interaction between type of washings and type of species in influencing the mean total bacterial count ($p>0.05$). Two-way Anova data also revealed that the mean total bacterial count of *periplaneta americana* was significantly higher than that of *blatella germanica* ($p<0.05$). In conclusion, all cockroaches harboured pathogenic bacteria with multidrug resistance, this means that cockroaches play a potential role in the epidemiology of nosocomial infections in this hospital.

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DEDICATIONS

I sincerely dedicate this body of work to my loving sister (Milly Makumana) and husband (Lookout Sigodo) who see the best in me and the heights of greatness I shall reach, even when I do not see it myself.

To succeed, you need to find something to hold on to, something to motivate you, something to inspire you –Tony Dorsett

TABLE OF CONTENTS

CHAPTER 1: INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Cockroaches are amongst the most common pests in urban environmental settings that are closely associated to food. There are known to carry and spread antimicrobial resistant bacterium frequently (Bennett and Owens, 2012). They are largely found in multi-family dwellings, and may act as carriers of several microorganisms affecting public health (Wood et al.,2010).Cockroaches are insects in the order Blatodea or Blaterria. The most important cockroaches of medical importance are *Periplanetta americana* (the American cockroach) *Blattella germanica* (the German cockroach), *Blatta orientalis* (the Oriental cockroach), *Supella longipalpa* (the Brown-banded cockroach). There are other many types of species and only four of these species are pests in homes, hospital settings and even office spaces. Cockroaches are of greater medical importance as they harbour a number of pathogenic and non-pathogenic micro-organisms (Salehzadeh *et al.,* 2007).

Most Third world countries like Zimbabwe, Angola, Malawi and Botswana suffer from limited resources, and this has resulted in the emerging rate of life threatening infections in health care facilities caused by these mechanical vectors (WHO, 2015). Third world countries are often known to lack the public surveillance system for detection of these acquired infections caused by cockroaches. Since the hospital environment provide cockroaches with suitable temperature, humidity and food, these insects are always present in variable numbers thereby resulting in their spread of infectious pathogens as proxy to Health Acquired Infections (Akhtar, 2010).

In hospital environments cockroaches are most prevalent in kitchens, intensive therapy zones, operating theatres and canteens. These creatures have been known to feed readily on faeces, sputum, skin scrapings and other contaminants (lslam *et al*., 2016). Their nocturnal habits make them ideal carriers for transmitting various pathogenic micro-organisms such as *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Proteus* species and *Klebsiella species, Actinobacter spp*. Cockroaches are known to derive their nourishment from vomit, phlegm from patients, food residues, excrements and other food sources (Kassiri, 2014).

According to Ducel *et al*., (2012) of the World Health Organization it has been noted that about approximately 10 million people are infected with nosocomial infections each year

especially in public hospitals due to the presence of mechanical vectors such as houseflies ,cockroaches and poor sanitation (Ducel *et al.*, 2012) defined a health acquired infection or nosocomial infections as infections that develop in a hospitalised patient or any other health care facility in which the infection was absent or incubating at the time of admission. Health care associated infections such as severe acute respiratory syndrome, urinary tract infections have brought about the immediate need for efficient infection control strategies to be put in place. These infections are preventable through implementation of best infection prevention and control practices especially at health care facilities (Ahaduzzaman *et al*., 2014).

Healthcare facilities are known to be the ideal settings for the transmission of infections to patients (who are more susceptible), healthcare workers, their families and communities who visit the hospital facility. Most healthcare associated infections lead to prolonged hospital stays, increased costs of care and death (Hsuch *et al*., 2012). Some of the most important bacteria to be analysed due to its resistance to antibiotics is a gram positive bacteria found in the family *Staphylococcae (*Medveďová and Valík, 2012).

1.2 PROBLEM STATEMENT

Most developing countries in Sub Saharan Africa suffer from limited resources, therefore the emerging rate of life threatening infections at health care facilities. Limited data, often of low quality, are available from low and middle income countries (Abdelkhalek *et al*.,2016). However, recent analysis by World Health Organization found that health care-associated infections are more frequent in resource limited settings than in developed countries (WHO, 2015). The ratio of health acquired infections is now higher in public hospitals than in private hospitals, this may be due to poor hygienic standards leading to an emergency of pests such as cockroaches (WHO, 2015). Some studies have also reported that cockroaches found in hospitals are playing important role in deteriorating hospital environment and patient's health by imposing stress, infections, anxiety, asthma, allergy in persons who have deficient immunity. Cockroaches have been found in hospital vicinity inside intensive therapy zone, neo natal units, ICU, medical wards, surgical wards and canteen, kitchen and medical stores (Sabra and Abdel-Fattah, 2012).

Sub Saharan African countries such as Zimbabwe, Botswana and Mozambique have given little attention to the spread of health acquired infections and the existence of such pestilence pests (Ducel *et al*.,2012). In 2012 statistics stated that up to 54 % of isolates from hospital environments were found to be human pathogens worldwide (Medveová and Valík, 2012). At

any given time, the prevalence of health care associated infection varies between 5.7% and 19.1% in low and middle income countries (Ducel *et al*.,2012). The proportion of patients with ICU-acquired infection ranged from 4.4% to 88.9% with a frequency of overall infections as high as 42.7 episodes per 1000 patient days in low and middle income countries such as Zimbabwe. This is almost three times higher than in high income countries such as Britain, America and Russia to name a few (WHO, 2015). Furthermore, in some developing countries, the frequency of infections associated with the use of central lines and ventilators and other invasive devices can be up to 19 times higher than those reported from Germany and the USA (Sausa *et al.,* 2014).

New- born babies are also at higher risk, with infection rates in developing countries 3-20 times higher than in high income countries. Among hospital born babies in developing countries, health care-associated infections are responsible for 4% to 56% of all causes of death in the neonatal period, and 75% in South-East Asia and Sub-Saharan Africa (WHO, 2015).

Surgical site infection and wound infections is the leading infection in the general patient population in countries with limited resources, affecting up to two third of operated patients and with a frequency up to nine times higher than in developed countries (Oliva *et al*., 2010). Several factors that lead to the prevalence of contaminants such as cockroaches harbouring bacteria are more specific to settings with limited resources and these are inadequate environmental hygienic conditions and waste disposal; poor infrastructure; insufficient equipment; understaffing; overcrowding; poor knowledge and application of basic infection control measures; lack of knowledge of injection and blood transfusion safety and the absence of local and national guidelines and policies. All these factors therefore lead to the emerging rates of cockroaches infestation (Medveďová and Valík, 2012).

Studies made in Botswana and worldwide have also shown that cockroaches are associated with a number of pathogenic and non-pathogenic organisms therefore may lead in transmission then it results in higher costs of medication for patients, health workers and the public, cockroaches have also been found to cause asthma allergies, prolonged hospital stay for operated patients due to post operational infections and the increase of antibiotic resistance of bacteria such as staphylococcus species. In Zimbabwe many people have limited access to medication due to impoverishment therefore they may eventually die of such infections (Mpuchane, 2006).

1.3 JUSTIFICATION

Cockroaches are known to carry 151 different species of bacteria and these in turn cause infections, it is therefore important to lower their rate of proliferation and enhance efficiency of infection control practices in hospitals and residential homes (lslam *et al*.,2016). As is the case for many other patient safety issues, health care-associated infections create additional suffering and come at a high cost for patients and their families. Infections prolong hospital stays, create long-term disability, increase resistance to antimicrobials, represent a massive additional financial burden for health systems, generate high costs for patients and their family, and cause unnecessary deaths. Such infections annually account for 37 000 attributable deaths in Europe and potentially many more that could be related, and they account for 99 000 deaths in the USA (Kassiri *et al*.,2014).

Annual financial losses due to health care-associated infections are also significant: they are estimated at approximately €7 billion in Europe, including direct costs only and reflecting 16 million extra days of hospital stay, and at about US$ 6.5 billion in the USA (Ducel *et al* .,2012). Financial costs attributable to health care-associated infections are poorly and variably reported in low and middle income countries (Pai, 2012).

For instance, the economic burden of health care associated infections in Belo Horizonte, Brazil, was estimated to be equal to US$ 18 million in 1992. In Mexican ICUs, the overall cost of one single health care-associated infection episode was US$ 12 155. In several ICUs in Argentina, the overall extra-cost estimates for catheter-related bloodstream infection and health care-associated pneumonia averaged US$ 4 888 and US$ 2 255 per case, respectively (Doosti *et al*., 2015). The emergence and the rapid spread of antimicrobial resistance bacteria such as *S. aureus* has become a global concern. Multidrug resistant (MDR) Staphylococcus poses a growing problem for human health and has been considered as horrifying public health threat (Ahaduzzaman *et al*., 2014).

Nosocomial infections in Zimbabwe can be reduced by : identifying local determinants of the Health Control System of Acquired Infections burden; improving reporting and surveillance systems at the national level; ensuring minimum requirements in terms of facilities and dedicated resources available for Health Control System of Acquired Infection surveillance at the institutional level, including microbiology laboratories' capacity; ensuring that core components for infection control are in place at the national and health care setting

levels; implementing standard precautions, particularly best hand hygiene practices at the bedside; improving staff education and accountability; conducting research to adapt and validate surveillance protocols based on the reality of developing countries and conducting research on the potential involvement of patients and the food they are given in that particular health environment, conducting national spraying day to reduce the number of mechanical vectors in all hospitals around the country(Oliva *et al*.,2010).

This project since it has never been undertaken here in Zimbabwe may be able to act as an awareness prospect to raise safety concerns in terms of cockroaches as pests. Safety awareness programmes may reduce the number of people being admitted daily in hospitals thereby lowering medication costs and morbidity and mortality caused by health acquired infections and a deeper understanding of cockroaches to the nation may also help in the reduction in antibiotic resistance of some bacteria such as *Staphylococcus*. Study of cockroaches and their association with the hospital environment may help clinicians in eradication of post surgery infections, neonatal infections, Urinary tract infections and life threatening septicaemia (Ahaduzzaman *et al*.,2014).

1.4 PROJECT OBJECTIVES

1.4.1 Main objectives : To investigate cockroaches as vectors of bacteria and to determine antibiotic susceptibility of acquired bacteria in a health environment as proxy to Hospital acquired infections.

1.4.2 SPECIFIC PROJECT OBJECTIVES

- To isolate and identify the types of bacteria harboured by *Periplaneta americana* and *Blatella germanica*
- To determine the possible role of cockroaches in the epidemiology of health acquired Infections.
- To enumerate the bacteria that are found in the external and internal washings of the two species of cockroaches *P.americana* and *B.germanica.*
- To investigate antibiotic susceptibility of bacteria isolated from cockroaches.

CHAPTER 2

LITERATURE REVIEW

2.1 GENERAL DESCRIPTION OF COCKROACHES

Cockroaches are members of the order Blattodea and they belong to the kingdom Animalia, the phylum is Arthropoda, the class is insect and the superorder is dityoptera. Cockroaches are brown insects with an antenna and are about one and a half inches (4 centimeters) long when fully grown. Cockroaches have a relatively small head and a broad, flattened body most are reddish brown to dark brown which includes the termites (Gordh and Headrick, 2009).

The body of cockroaches is divided into a thorax of three segments and a ten-segmented abdomen. The external surface has a tough exoskeleton which contains calcium carbonate and protects the inner organs and provides attachment to muscles (Hoell *et al*.,1998). It is coated with wax to repel water. The wings are attached to the second and third thoracic segments. The tegmina, or first pair of wings, are tough and protective, lying as a shield on top of the membranous hind wings, which are used in flight. All four wings have branching longitudinal veins, and multiple cross-veins (Legentre *et al*., 2015).

Cockroaches also have a long antennae and legs, feeding by scavenging. The three pairs of legs are sturdy with large coxae and five claws each. They are attached to each of the three thoracic segments(Mullen and Burden, 2002).The front legs are the shortest and the hind legs the longest, providing the main propulsive power when the insect runs .Cockroaches are insects, flattened from top to bottom, usually with two pairs of wings folded flat over the back. Most species rarely fly but they move very fast using legs. The colour usually varies from light brown to black. The species vary from 2– 3mm to over 80mm in length, of over 3500 identified species only a few are of importance to people because they have adapted to living in buildings(Sweld, 2015).

2.1.1 THE BIOLOGY OF COCKROACHES

The most common species are: *Periplaneta americana*, the American cockroach, which occurs around the world. It is 35 to 40mm in length and has a shiny reddish to chocolate brown colour. The egg case measures 8 to 10mm and contains 16 eggs (Bell *et al*.,2011) .*Periplaneta australasiae*, the Australian cockroach, which occurs mainly in tropical and subtropical areas. It is similar to the American cockroach, but smaller (31 to 37mm long) and

darker. It has a pale yellow stripe on each forewing extending for about one-third its length. The egg case contains about 22 to 24 eggs (Copeland, 2004).

Blatta orientalis, the Oriental cockroach, found mainly in cool temperate regions. It is blackish and 20 to 27mm long .The egg case is 10 to 12mm long and contains 16 to 18 eggs(4) *Supella longipalpa*, the brown-banded cockroach, which occurs around the world. It is 10 to 14mm long and has yellow and brown bands. The egg case is 4 to 5mm in length and contains about 16 eggs (Bell *et al*., 2007). *Blattella germanica*, the German cockroach, found in most parts of the world. It is light yellowish brown and 10 to15mm in length, making it one of the smallest domestic cockroaches. The female usually carries the egg case until shortly before the young come out. The egg case is light in colour, about 7 to 9mm long and contains about 40 eggs (Khalaji *et al*., 2013). The other examples of cockroaches are Argentinian (*Blattaria sudamerican*), Asian(*Blattella asahinai*), Australian(*Periplaneta australasiae*), Brown-banded(*Supella supellectilium*), Dusky-brown(*Periplaneta fuliginosa*) and Madeira (*Leucophaea madera).*

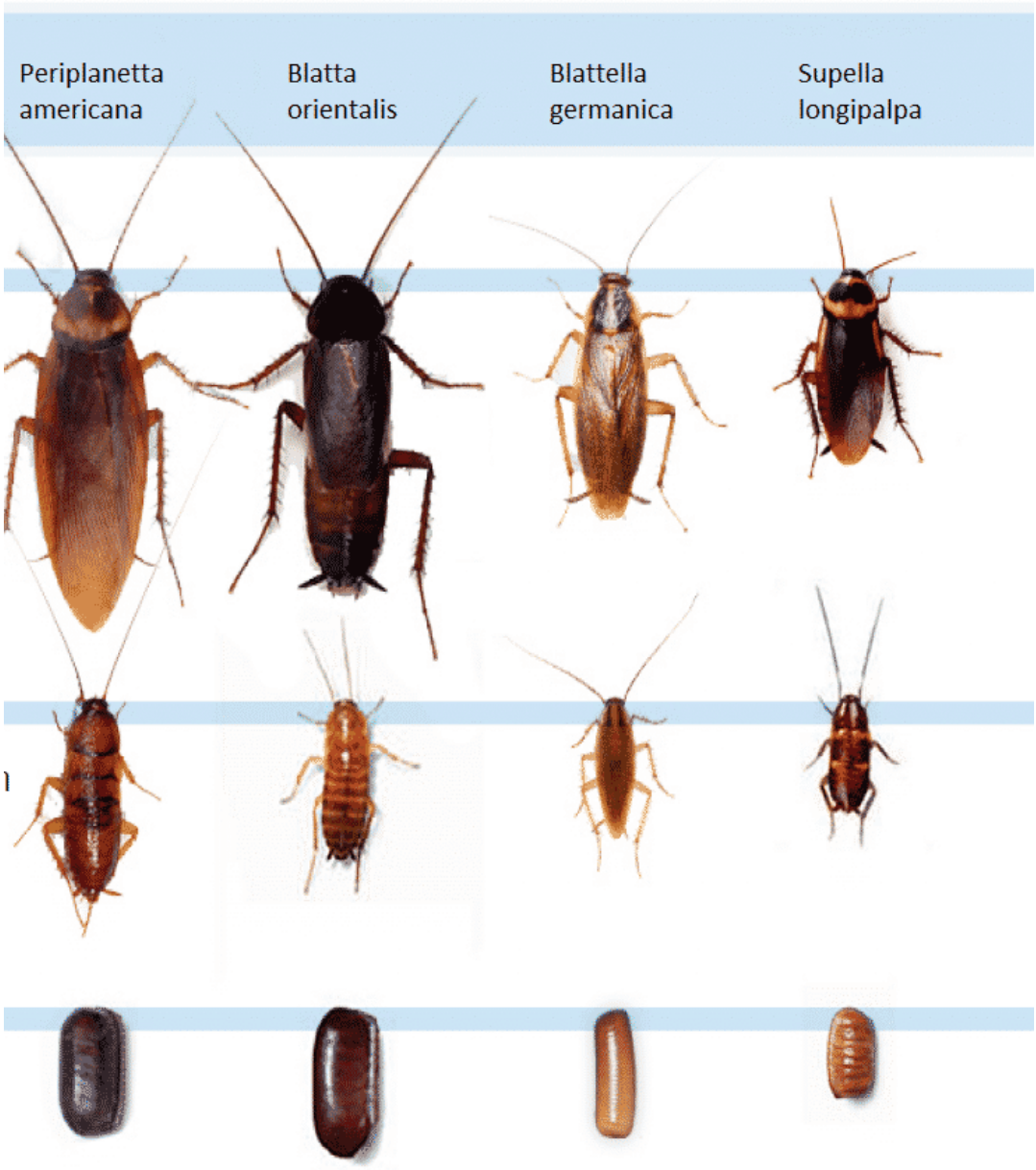


Figure 2.1 Common household cockroaches (a) *Periplaneta americana*,(b) *Blatta orientalis* (c) *Blatella germanica* (d) *Supella longipalpa*.

Cockroaches are relatively primitive, having only three stages in their life cycle: egg, nymph and adult. The female deposits its eggs in groups surrounded by a leathery, bean-shaped egg case or capsule called an ootheca. Some species, such as the German cockroach, carry the ootheca for several weeks attached to the back end of the body. Most others deposit the

ootheca after one or two days. Oothecae are very distinctive and can frequently be used to determine the species present (Beccaloni and Eggleton, 2013).

Depending on the species, temperature and humidity, the eggs hatch after 1–3 months. The young cockroaches, or nymphs, are wingless, and usually only a few millimetres long; they are white on hatching but darken within a few hours. They grow in stages by repeatedly shedding the cuticle or skin (Siachua *et al*., 2008). They are fully grown after several months to more than a year, depending on the species. The adults may or may not possess wings, consisting of one outer leathery pair beneath which is folded a membranous pair (Huang *et al*., 2012)

2.1.2 ECOLOGY AND BEHAVIOUR OF COCKROACHES

Cockroaches are known to play a supplementary role in the spread of some diseases. Cockroaches are the most significant and objectionable pests found in apartments, homes, food-handling establishments, hospitals, and health care facilities worldwide. They are among the most common pests in many homes and other buildings (Cornwell, 2005) Cockroaches consume garbage, rotting food, and even faecal waste of other cockroaches. They then transmit disease to your food, eating utensils, kitchen surfaces, and other areas around your home. They can easily contaminate food by leaving droppings which may contain bacteria that can cause food poisoning, fungi, and other pathogenic organisms (Malcom, 2013). Their nocturnal and filthy habits make them also ideal carriers of various pathogenic microorganisms. At night they search for food in kitchens, food storage places, rubbish bins, drains and sewers. Indoor species, especially the German cockroach, exploit conditions associated with high-density human populations and impoverished living conditions. Cockroaches are largely found in multi-family dwellings, and may act as a carrier of several microorganism affecting public health (Jeanson *et al*., 2005).

Cockroaches are proven or suspected carriers of the organisms causing: diarrhoea, dysentery, cholera, leprosy, plague, typhoid fever and other viral diseases such as poliomyelitis. In addition they carry the eggs of parasitic worms and may cause allergic reactions, including dermatitis, itching, swelling of the eyelids and more serious respiratory conditions Botella,2005: Oliva *et al*.,2010.

Their nocturnal lifestyle and dirty habits make them ideal carriers for transmitting numerous pathogenic bacteria, such as *Listeria monocytogenes, Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa, Shigella species, proteus sp, Streptococcus species.,*

Salmonella species, Campylobacter species, Escherichia coli, and other potential pathogens (Salehzadeh *et al*., 2007; Kassiri *et al*., 2014; Doosti *et al*., 2015. ln a study done in Botswana More than 33.3% of cockroach isolates have shown resistance to antimicrobials (Mpuchane *et al*., 2006). *Staphylococcae* is responsible for the skin infections which then can manifest in various ways like boils, cellulitis and more severe, invasive soft tissue infection. *Staphylococcus* is resistant to many commonly used antibiotics methicillin resistant *Staphylococcus aureus*, Vancomycin intermediate *Staphylococcus aureus* and Vancomycin resistant *Staphylococcus aureus*. Secondly Gram positive bacteria e.g. *Staphylococcus aureus* is a cutaneous bacteria that colonize the skin and nose of both hospital staff and patients cause a wide variety of lung, bone, heart and blood stream infections and are frequently resistant to antibiotics; beta-haemolytic streptococci are also important (Cheesbrough, 2006).

2.1.3 THE PATHOLOGY OF COCKROACHES

Cockroaches are known to transmit up to nine different diseases resulting from their contamination on food and water. In addition to bacteria, many species of parasites were isolated from external surface and guts of cockroaches included cysts of *Entameoba coli*, *Entameoba histolytica* and *Giardia lamblia* and adult and ova of *Enterobius vermicularis* and ova of *Hymenolepis nana* and *Ascaris lumbricoides* (Wannigama, 2014) It was also proved experimentally that cockroaches may play an important role in the transmission of helminthes. Some of the diseases include Salmonellosis: cockroaches similar to rodents are known to transmit the *Salmonella* bacteria which can cause *salmonellosis*, which is a disease in humans with symptoms similar to food poisoning. Cockroaches are known to accumulate the bacteria by feeding on contaminated food. *Salmonella* remains in their digestive system for a month or more and is deposited through their vomit and faeces (Rentz,2014).

Typhoid fever is another type of a bacterial infection caused by the *Salmonella typhi* bacterium and is a highly infectious disease. It is believed that cockroaches accumulate this disease by consuming faeces contaminated with the bacterium. Children and the elderly are thought to be most at risk due to their immunocompromised state (Wu Hao, 2013).

Cholera is an acute diarrhoeal infection caused by the *Vibrio cholerae* bacterium. It is most common in developing countries and areas that have inadequate environmental management. Infection occurs through ingestion of food and drink contaminated with the bacterium (Gilliot, 2003). If exposed to the bacterium, cockroaches can spread the organism through their faeces and vomit, contaminating surfaces and food. Researchers from the World Health

Organization have estimated that worldwide there are roughly 1.4 million to 4.3 million cases of cholera per year resulting in 28,000 to 142,000 deaths (WHO,2015).

Dysentery is a type of gastroenteritis that results in diarrhoea with blood. Generally, most people suffer from mild symptoms and recover within a week or so without medical attention. There are two types of dysentery. They are: Bacillary dysentery: Sometimes referred to as shigellosis. It is caused by the *Shigella* bacteria. Amoebic dysentery: Is caused by a single-celled parasite called Entamoeba. It is usually found in tropical areas (Gulani *et al*., 2016).

Campylobacteriosis is an infection caused by the Campylobacter bacterium. It is one the most common bacterial infections in humans, and is a common foodborne illness. Transmission occurs through ingesting contaminated food and drink such as unpasteurised milk and undercooked and poorly handled poultry. Researchers have isolated a *Campylobacter jejuni* subspecies in the gut contents and on the external surface of both American cockroaches and Oriental cockroaches (Umunnabuike and Irokanulo, 2000) .*Escherichia coli (E. coli)* is a bacterium commonly found in the gut of humans. Although most strains of the bacteria are harmless, some can cause serious food poisoning. The common symptoms of an *E. coli* infection are watery diarrhoea and abdominal cramping. Less common symptoms are: Fever, Chills, Nausea and Muscle aches (Guarino, 2016).

Cockroaches can trigger asthma because they certain proteins in their bodies which can be an allergen for certain people. When tiny particles from cockroach bodies are spread through the air in buildings, these proteins are inhaled and an asthma attack can be triggered in sensitive people. The American College of Allergy, Asthma & Immunology reports that the saliva, faeces and shed skin of cockroaches can trigger both asthma and other allergic responses (Botella *et al*., 2005).

2.2 ENVIRONMENTAL MANAGEMENT

Cleanliness and hygiene should be maintained in the hospital area and leftover food should be stored in tightly covered containers in clean cabinets or refrigerators. All areas have to be kept clean so that no fragments of food or organic matter remain. Rubbish bins should be securely covered and emptied frequently, preferably daily. Effective control is easier in temperate climates (where cockroach populations cannot survive outdoors in winter) than in humid and warm areas. The key to control is cleanliness, which may be difficult in houses where there are children and domestic animals (Gilliot, 2003). In isolated homes, control is

easier to achieve than in apartments where cockroaches may have easy access from adjacent quarters. Reinfestation occurs from outdoors in warm areas, or along heating ducts and water pipes in apartments, or from groceries or luggage brought from cockroach infested areas (Gliniewicz, 2003). Cockroaches may even sometimes be found in very clean houses, but are unlikely to establish colonies. The presence of several sizes of nymphs and oothecae is an indication of a well established colony. Infestations can be detected by searching behind skirting boards, boxes, furniture and other common hiding places. At night, cockroaches are easily detected using light (Rentz , 2014).

2.3 HOSPITAL ACQUIRED INFECTIONS

According to (Ducel *et al*., 2012) A hospital acquired infection also known as a nosocomial infections is defined as 'An infection acquired in hospital by a patient who was admitted for a reason other than that infection or An infection acquired by a patient in a hospital or any other hospice in whom the infection was absent or incubating at the time the patient was admitted. These infections can even start to appear even after the patient has been discharged, and hospital acquired infections can also manifest among the staff of the facility/health workers (Manott and Gravani, 2006). In Zimbabwe the care of many patients is provided in facilities which range from highly expensive and equipped clinics and technologically advanced hospitals such as Baines hospital to front-line units with only basic facilities, but despite that the rate of Health Acquired Infection keeps on increasing (WHO, 2015). Despite progress in public health and hospital care, infections continue to develop in hospitalized patients, and may also affect hospital staff. There are also several factors that promote infection among hospitalized patients such as decrease in immunity among patients due to several diseases; the increasing variety of medical procedures and invasive techniques creating potential routes of infection; and the transmission of drug-resistant bacteria among crowded hospital populations, where poor infection control practices may facilitate transmission (Huang *et al.*, 2012).

Bacteria are the most common nosocomial pathogens Gram-negative bacteria: Enterobacteriacae (e.g. *Escherichia coli, Proteus, Klebsiella, Enterobacter, Serratia marcescens),* may colonize sites when the host defences are compromised (catheter insertion, bladder catheter, cannula insertion) and cause serious infections (surgical site, lung, bacteraemia, peritoneum infection) .They may also be highly resistant (Pavlik and falkinham, 2009).

Gram-negative organisms such as *Pseudomonas spp*. are often isolated in water and damp areas. They may colonize the digestive tract of hospitalized patients. Selected other bacteria are a unique risk in hospitals. For instance, *Legionella species* may cause pneumonia (sporadic or endemic) through inhalation of aerosols containing contaminated water (air conditioning, showers (Feizhaddad *et al*., 2012).

2.3.1 URINARY INFECTIONS

Cockroaches due to their nocturnal movements acts as mechanical vectors in contaminating catherised devices in search for food. Catherised patients especially those with the device in situ for long periods experience repeated episodes of infections that can damage the urinary tract. (Bennet, 2010). Urinary infections forms the most common nosocomial infection; according to recent records there are about 80% of infections which are associated with the use of an indwelling bladder catheter (Fox, 2008). Although there are highly common most of the urinary infections are associated with less morbidity than other nosocomial infections, but although at a lesser extent can occasionally lead to bacteraemia and death. The bacteria responsible arise from the gut flora, either normal (Escherichia coli) or acquired in hospital (multiresistant Klebsiella). Catherised patients especially those with the device in situ for long periods experience repeated episodes of infections that can damage the urinary tract (Mullen and Durden, 2002).

2.3.2WOUND INFECTIONS

The skin is highly known for its use as a barrier for many microorganisms as they prevent entry of bacteria entering the body (Bouamama *et al*., 2010). Most cockroaches move on devices and cloth used in cleaning of wounds thereby leaving bacteria which can then cause infections on wounds. Wounds often are a result of a trauma that occur to people through accidents ,surgery, burns, certain infectious opportunistic diseases and other various ulcerations. Culture can be used in identification of organisms causing infection by use of wound swab (Khalaji *et al*., 2013).Burns are most problematic especially in those patients having burns affecting more than 60%of the surface of the body are more susceptible with 70% of these burns becoming infected and most patients developing blood stream infections. Gram negative bacteria such as *Proteus* species are common (Cheesbrough, 2006).

3.0 MATERIALS AND METHODS

3.1 STUDY SITE

The sampling area of this study was a Health care facility in Gweru. The experiments were carried out at Gweru General Hospital Laboratory. The health care facility has a population of approximately 20 patient care units. The Sample size of this study were 2 hospital units' the hospital kitchen and the canteen. In this study our sampling strategy was a convenient sampling. Sampling step – the first cockroaches for inclusion in this study were collected at the following patient care units –the hospital main kitchen and the canteen kitchen and were then transferred to the hospital laboratory for further research.

3.2 Sample Collection

The simple jar traps method was used in the collection of the required cockroach specimens. Bread crumps were placed at the bottom of the container to act as attractants and a thick layer of petroleum jelly was added on the inner rim of the container to prevent the insects from escaping. Thirty- four cockroaches were collected, over a period of 1 month,16 from the health main kitchen and 18 from the canteen. The cockroaches were collected in late day time. After a two day observation the trapped cockroaches were collected in sterile test tubes, transported to the laboratory and anaesthetized by freezing at 0^0C for 5 minutes and then stored at 5^0C. The cockroach differences were compared using their morphological features (Harwood and James, 1979).

3.3 THE IDENTIFICATION AND ISOLATION OF MICROORGANISMS FROM THE COCKROACHES ON THEIR EXTERNAL SURFACES.

Following the morphological identification of each of the cockroaches, two ml of sterile normal saline was added in each tube and the cockroaches were thoroughly shaken for two minutes. The cockroaches were then taken out of the tubes and the remaining liquid containing bacteria was centrifuged at 2000 rpm for 10 minutes. The supernatant was then removed and the remaining sediment was used for culture. From each tube a fixed volume of 1 ml each of the washings was cultured on selective media MacConkey agar, mannitol salt, nutrient agar and blood agar plates separately. Culture plates with different culture media were labelled ES(external surface) , P (*periplaneta americana)* and the sample number, the labels were put in this order on the plates P, E.S and 1. The same procedure was done for

external washings of *Blatella germanica* each plate were also labelled ES,S (*Blatella germanica*) and 1 .The cultures were incubated overnight at 37 ^{o}C. The ultimate colonies were then identified by standard bacteriological procedures (Cheesbrough, 2006).

3.3.1 ThE identification and isolation of microorganisms from the cockroaches on their internal surfaces.

Following the external washings culture, the external washings surface of cockroaches were then washed with 70 percent ethyl alcohol for 5 minutes and were then allowed to dry at room temperature under sterile conditions. The decontaminated cockroaches were then washed with sterile normal saline for 2-3 minutes to remove traces of alcohol. The gut of the cockroaches were dissected out and macerated aseptically with a sterile pestle and mortar in 2 ml of sterile normal saline. The resulting macerate were cultured on selective media at 37^{o}C overnight and in a similar way as described above and the results were recorded.

3.4 MICROSCOPIC IDENTIFICATION

Gram staining was done. All the positive samples of gram staining were subjected to coagulase and catalase tests for biochemical confirmation as described by Cheesbrough (2006).

3.5 Biochemical Tests

Biochemical tests were performed on colonies from primary cultures for identification of the isolates. Gram-negative rods were identified by performing a series of biochemical tests such as Kligler iron test, citrate utilisation test (SC), Lysine decarboxylase test, indole motility test, urease test, coagulase test, catalase test and oxidase test. Gram-positive cocci were identified based on their gram reaction, catalase, coagulase and oxidase test results.

3.5.1 CATALASE TEST

Two drops of 3% hydrogen peroxide (H_2O_2) were added into a test tube using a dropper. An isolated bacterial colony from the MacConkey agar was placed in the drop of hydrogen peroxide by using a sterilised inoculation loop sterilised by dipping it in methylated spirit, flaming it until red hot and then allowing it to cool in the air. The drop was viewed for any effervescence.

3.5.2 OXIDASE TEST

A manufactured oxidase test strip was inoculated with an isolated bacterial colony using a sterilised inoculation loop. The test part of the strip was viewed for any colour change.

3.5.3 CITRATE TEST

Simmons citrate agar was prepared by weighing 11g of the agar powder and dissolving in 250ml of distilled water. The solution was then mixed and heated until the powder was completely dissolved. The solution was then autoclaved at 121°C for 15minutes, and after that, it was poured into labelled bijou bottles to cool and set. Using a sterile straight wire, the slope was first streaked with a saline suspension of the test organism/colony type and then the butt was stabbed. The Simmons citrate agar was incubated at 35 ^{0}C for 48 hours. The medium was then observed for any colour change.

3.5.4 INDOLE TEST

Bijou bottles with peptone water were labelled with the test organism name and sample code. Gram negative lactose fermenting colonies were picked from the MacConkey agar plates using a sterilised inoculating loop and a suspension was made in the peptone water. These were then incubated at 370C for 24 hours and two drops of Kovac's reagent were added into each bottle. Samples were observed for the presence of a red ring or a brown ring and checked against positive and negative controls available in the laboratory then recorded

3.5.5 COAguLASE

Glass slides were labelled with the test organism and sample code. Sterile saline was inoculated onto the glass slides using a sterilised inoculating loop. A colony of the test organism was then emulsified onto the slides and a loopful (not more) of plasma was added to one of the suspensions, and mixed gently. Clumping of the organisms was observed within 10 seconds No plasma was added to the second suspension. This was used to differentiate any granular appearance of the organism from true coagulase clumping.

3.6 BACTERIOLOGICAL ANALYSIS USING QUANTITATIVE METHODS - BACTERIAL ENUMERATION

3.6.1 TOTAL BACTERIAL COUNTS

In order to determine the bacterial load of the two species of cockroaches the pour plate method was used. In this method, each sample cockroach was washed in 1% normal saline to form a broth and then removed from the solution. The sample container had a combination of American cockroaches *Periplaneta americana* and brown banded *Blatella germanica* from the two sampling areas and were fully labelled. The cockroaches were randomly picked each from a trap container with cockroaches from the canteen and kitchen using forceps and, then each one was transferred into a sterile dilution bottle containing 1% normal saline following aseptic techniques. The latter were then shaken vigorously by hand before use in the serial dilutions. In one tube l ml of undiluted broth of the cockroach was added into 9 ml of 1% normal saline this was labelled 10^{-1} .Further serial dilutions were made up to 10^{-4}.

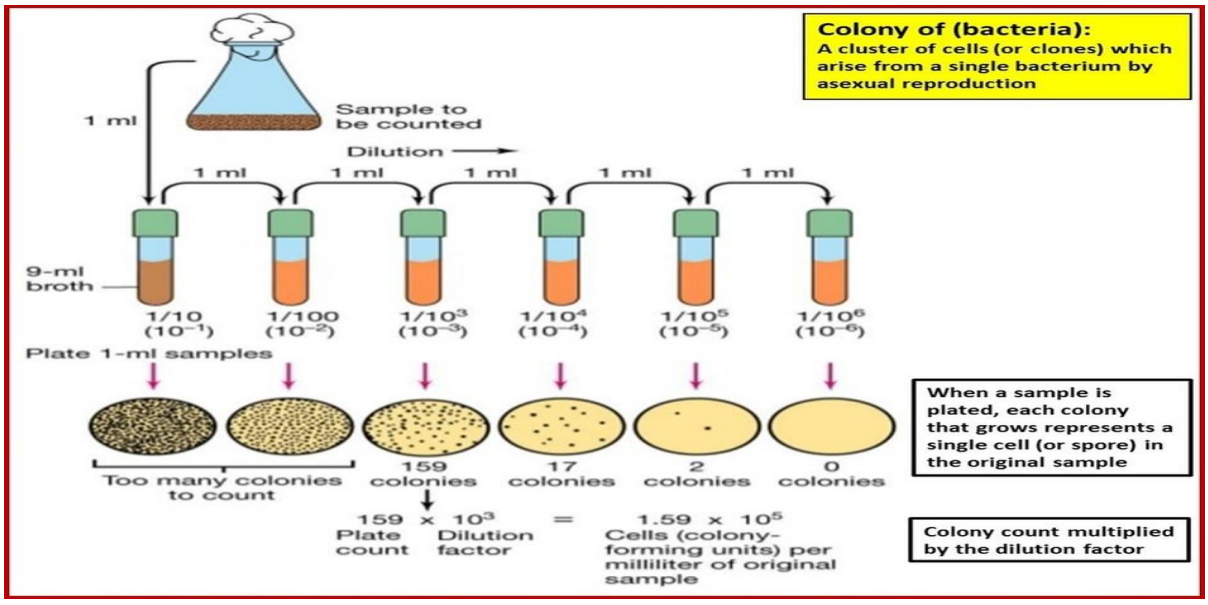


Figure 3.1-Diagram showing serial dilutions (Mullen and Durdan, 2002)

Lastly, a fixed amount of inoculum (1 ml) from each labelled diluted broth samples were then poured each into allocated petri dishes containing Plate count agar using the Pour plate method and then mixed well by swelling the culture plate. After the solidification of the agar, the plate was inverted and incubated at 37°C for 24-48 hours. Following incubation the plates were examined .The number of bacteria obtained was then counted on each plate using the bacterial colony counter. For each sample, the dilution which had growth of between thirty to

three hundred colonies were selected and the colonies were counted. For samples which had less than thirty colonies they were regarded as too little to count and those higher than 300 too high to count. The results were recorded and calculations of colony forming units per millilitre (cfu/ml) were done as follows;

Total bacterial Count (Cfu/ml) = number of colonies X the dilution factor

3.6.2 Total ColifoRm count

In Total coliform counts the same procedure used in Total bacterial counts was used except the type of media used to grow the bacteria was MacConkey agar.

3.7 ANTIBIOTIC SUSCEPTIBILITY TESTS

Isolated bacteria were tested for antimicrobial susceptibility using Mueller Hinton agar with Kirby Bauer disk diffusion method using :for gram positives, gentamicin (10 µg),amoxicillin 30 µg, ciprofloxacin 5µg, chloramphenicol 30µg,streptomycin 30 µg, Augmentin 25 µg and erythromycin15µg and :for gram negative gentamicin 10 µg, amoxicillin 10 µg, chloramphenicol 30 µg ,streptomycin 30 µg and erythromycin 15 µg. Inhibition diameters were measured and interpreted according to the standard interpretive zone size chart of the manufacturer.

3.8 STATISTICAL ANALYSIS

Rates of isolation bacteria in antibiotic susceptibility tests were compared. A P value of less than 0.05 was considered to be statistically significant. The Total bacterial counts conformed to normality (Shapiro Wilk) and were analysed using Two Way Analysis of Variances (two-way ANOVA), SPSS Package version 21.

CHAPTER 4 RESULTS

4.1 BACTERIAL IDENTIFICATION

In this study a total of five pathogenic bacteria were isolated from the cockroach samples .These suspected microorganisms included *E.coli* which formed pink colonies on MacConkey, *Staphylococcus aureus* formed large pin head size and golden yellow colonies on nutrient agar, *Proteus spp* formed gray smooth colonies with a fish odour on MacConkey, *Pseudomonas aeruginosa* formed clear colonies with a grape like odour and a non-lactose fermenter and *Klebsiella pneumonia* formed pink mucoid colonies on MacConkey.

TABLE 4.1 – Summary of Isolated bacteria from *periplaneta americana*

***P.americana* -internal washings**	***P. americana* –external washings**
E.coli	*Klebsiella pneumonia*
Klebsiella pneumonia	*Staphylococcus aureus*
Staphylococcus aureus	*E.coli*
	Proteus

TABLE 4.2- Summary of isolated bacteria from blatella germanica

***B.germanica* -Internal washings**	***B-germanica*-External washings**
E.coli	*Klebsiella pneumonia*
Klebsiella pneumonia	*E.coli*
Proteus	*Staphylococcus aureus*
Pseudomonas aeruginosa	*Pseudomonas aeruginosa*
Staphylococcus aureus	*Proteus*

4.1.2 Table 4.3 -Macroscopic Results

Type -of Colonies	Size	Colour	Elevation	Edge	Suspected bacteria
Colony A	Large pin head size colonies	Opaque golden growth on nutrient agar	Convexly elevated with smooth edges	Entire edge	Staphylococcus aureus
Colony B	circular and halo shape with entire smooth edges	pink colonies on MacConkey	umbonately elevated, raised in the center more than on the edges	Entire edge with a butyrous constistency	Escherichia coli
Colony C	Small irregular spreading /swarming colonies on nutrient agar	Thin blue grey smooth colonies	Convexly elevated with smooth undulate edges	Undulate edges	Proteus spp
Colony D	Tiny sized, colonies with a smooth glistening surface	Bright pink colonies on MacConkey agar	Low,Convexly Elevated with ,membranous constistency	Entire edges with a friable constistency	Klebsiella spp
Colony E	Large, opaque irregular colonies on Nutrient agar	Clear colonies with a fresh tortilla odour	Umbonately elevated, with irregular shape	Low undulate edges	Pseudomonas aeruginosa

4.1.3 Table 4.4

Biochemical tests results

Gram status	**Catalase test**	**Coagulase test**	**Simmons citrate test**	**Oxidase test**	**Indole motility test**	**Urease test**	**Kligler test**	**Suspected organism**
Gram positive cocci, grape like cocci	+	+	-	-	-	-		*S.aureus*
Gram-negative rods	+		+	-	+	+	Black butt observed	*Proteus spp*
Rod shaped coliform	+	-	+	-	+	+	Yellow slant throughout media	*Escherichia coli*
Moderate gram negative rods	+		+	-	-	+		*Klebsiella spp*
Gram negative rods	+	-	+	+	-	-	Alkaline slant; no change in butt	*Pseudomonas Auroginosa*

4.2 PREVALENCE OF BACTERIAL ISOLATES FROM DIFFERENT ANATOMICAL SAMPLING SITES

Thirty-two bacterial isolates were recovered from the cockroach samples and their treatments and percentage distribution of each genera of bacteria isolated are depicted below in Table 1; *Escherichia coli* showed highest frequency of 34.4%, followed by *staphylococcus aureus* with 21.9%, followed by *Proteus* spp with 18.8%, and the lowest were *Klebsiella pneumonia* with 12.5% and *Pseudomonas aeruginosa* with 12.5%.

Table 4.5- Prevalence of bacteria

BACTERIA	ISOLATES	PREVALENCE(%)
Escherichia coli	11	34.4%
Klebsiella pneumonia	4	12.5%
Staphylococcus aureus	7	21.9%
Proteus sp	6	18,8%
Pseudomonas aeruginosa	4	12,5%
TOTAL	32	100

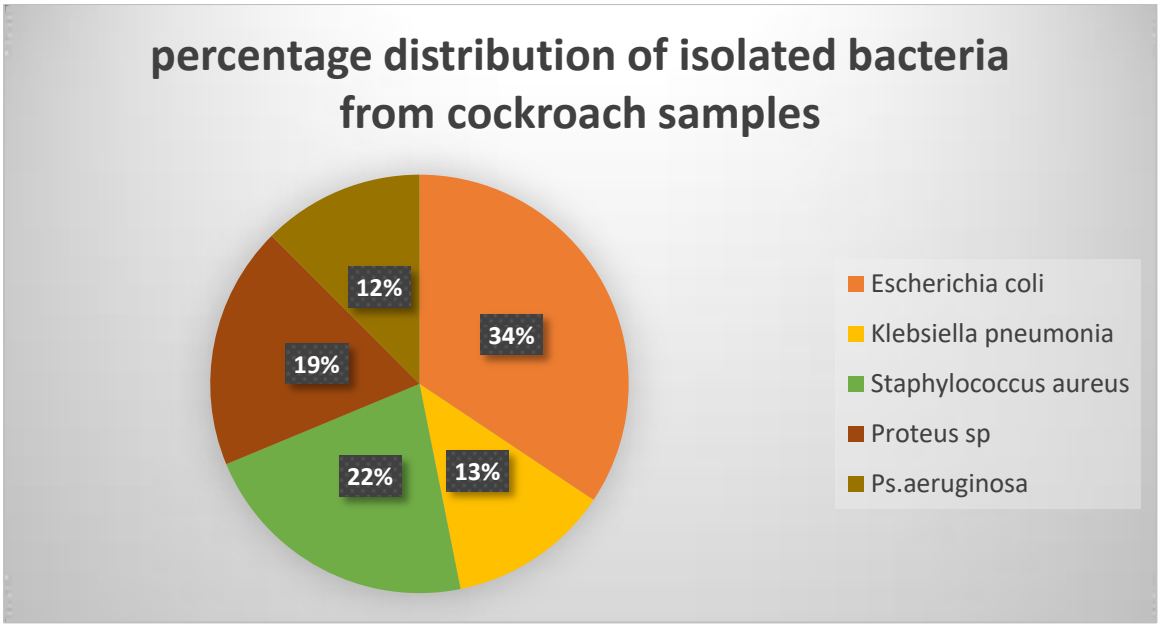


Figure 4.1 Pie chart showing the percentage distribution of bacterial isolates from cockroach samples.

It was observed that Escherichia coli has the highest prevalence of the isolated bacteria followed by *staphylococcus aureus*, followed by *Proteus sp* and lastly the lowest were *klebsiella pneumonia* and *Ps. Aeruginosa*.

4.3 QUANTITATIVE ANALYSIS –COLONY COUNT

4.3.1 TOTAL BACTERIAL AND TOTAL COLIFORM COUNTS

The bacterial assessment of cockroach samples found in a hospital in Gweru were examined and the mean total bacterial counts of the external washings of the *Periplaneta americana* ranged from 3.60×10^4 cfu/ml to 3.50×10^4 cfu/ml. The mean total coliform count ranged from 3.40×10^4 cfu/ml to 3.00×10^4 cfu/ml. The mean total bacterial count of the external washings of *Blatella germanica* ranged from 2.50×10^4 cfu/ml to 3.20×10^4 cfu/ml (Figure 4.2)The mean total coliform count ranged from 2.05×10^4 cfu/ml to 3.00×10^4 cfu/ml.

The mean total bacterial count of the internal washings of the *periplaneta americana* ranged from 3.00×10^4 cfu/ml to 3.59×10^4 cfu/ml. The mean total coliform count ranged from 3.0×10^4 cfu/ml to 3.05×10^4 cfu/ml. The mean total bacterial count of the internal washings of *blatella germanica* ranged from 2.55×10^4 cfu/ml to 3.23×10^4 cfu/ml. The mean total coliform count ranged from 2.10×10^4 cfu/ml to 2.85×10^4 cfu/ml (Figure 4.2).

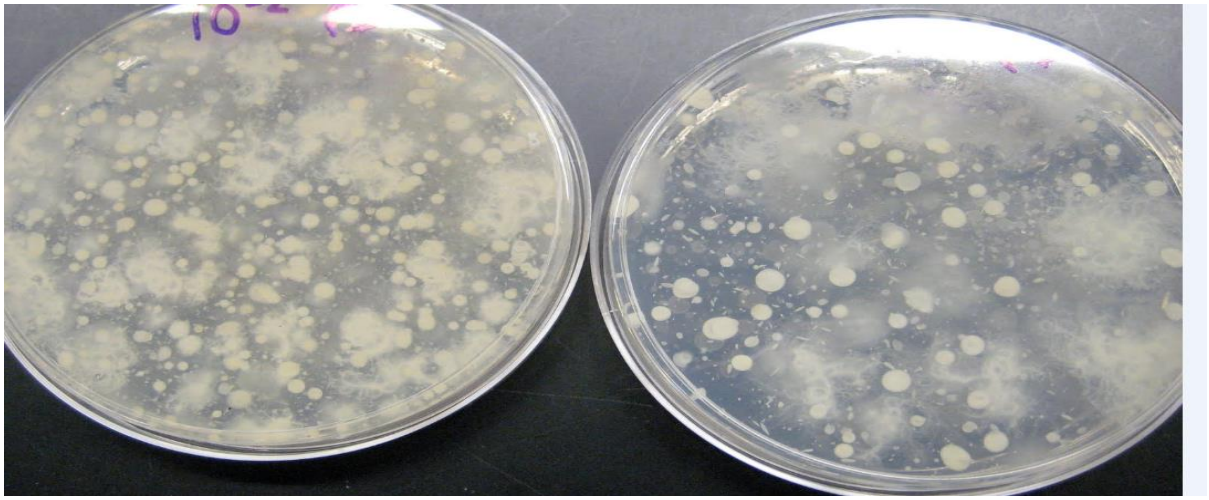


Figure 4.2 Bacterial counts plates

4.3.2 STATISTICAL ANALYSIS;

Amongst the thirty four cockroaches collected 32(94.1%) were found to carry one or more species of microorganisms either on their external surface or their internal surface. Data for Total Bacterial Count was tested for normality using Shapiro Wilk on SPSS version 21 and the data values and their error terms followed a normal distribution for each treatment combination ($p>0.05$.Appendix 5.1).

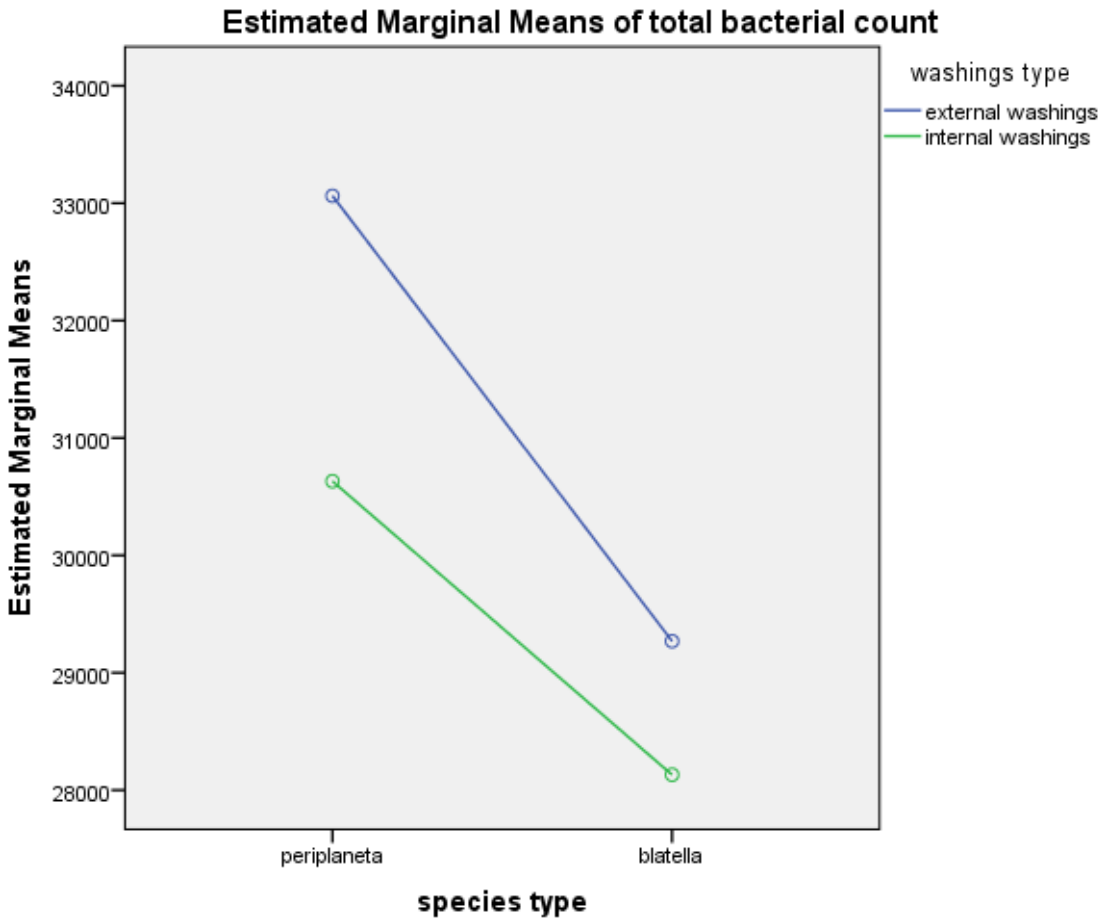


Figure 4.3Comparative marginal mean graph of the types of washings and species

Tests for homogeneity of variance was also performed using Levene's test of equality of error variance and the variance of data values and their error terms were equal across all treatment groups ($p>0.05$; appendix 5.2).

Two-way Anova results showed that there is no statistically significant interaction between type of washings and type of species in influencing the mean total bacterial count ($p>0.05$;appendix 5.3) .There was also no statistically significant difference in internal washings and external washings in influencing the mean total bacterial count ($p>0.05$;Appendix 5.3). The data also shows that there is a significant difference in the overall isolation rate of bacteria between the two species *periplaneta americana* and *blatella germanica*. The mean total bacterial count of *periplaneta americana* is significantly higher than that of *blatella germanica* ($p<0.05$;appendix 5.3). The data shows that the rate of isolation from the external surface was found to be significantly higher than that from the internal surface in *B. germanica* (P <0 .05;appendix 5.3).

In The data for Total colony count, data values were tested for normality using Shapiro-wilk and (p>0.05;appendix 6.1)therefore data values and their error terms followed a normal distribution .Data was also tested for homogeneity of variance using Levene's test of equality of variance and the data values and their error terms were equal across all treatment groups (p>0.05;appendix 6.2).Two-way Anova results showed that at 5 % significance level that there is no interaction between the type washing and species type in influencing Total Colony Count. (P.>0.05;appendix 6.3).This concludes that even though cockroaches are not themselves pathogenic they are mechanical carriers of pathogenic bacteria. The mean total bacterial count of the *periplaneta americana* is higher than that of *blatella germanica*, whereas the external surface washings of *periplaneta* is also higher than in *B.germanica*. Small Standard deviation bars on blatella germanica internal washings therefore low spread, data are clumped around the mean.

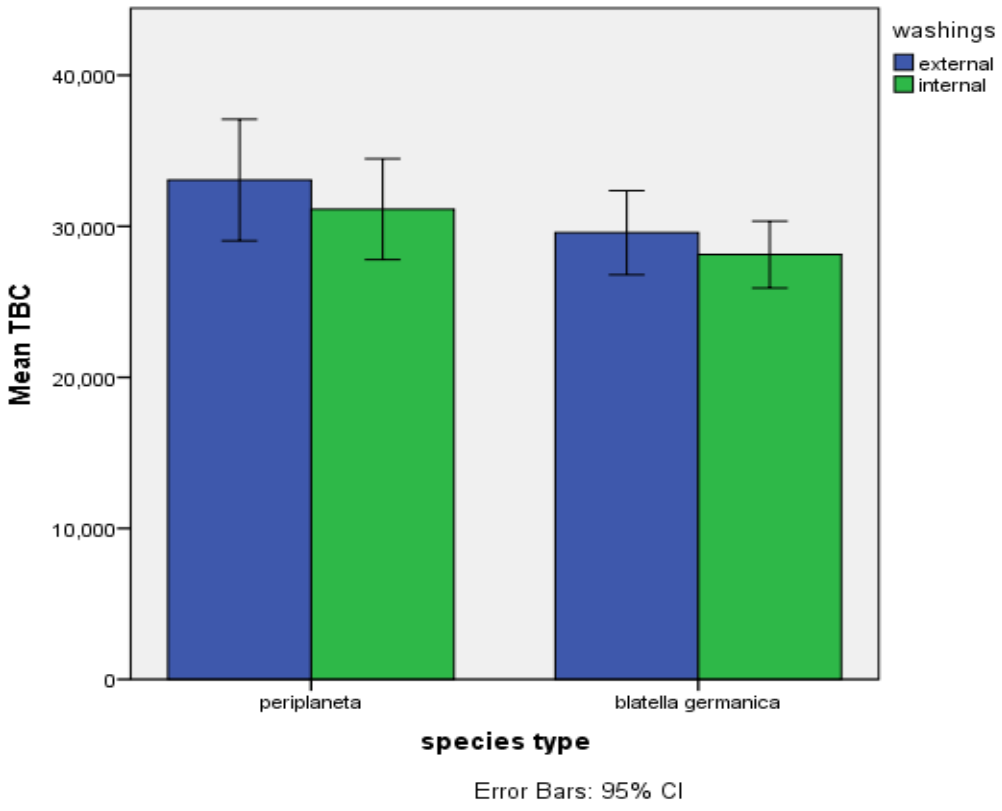
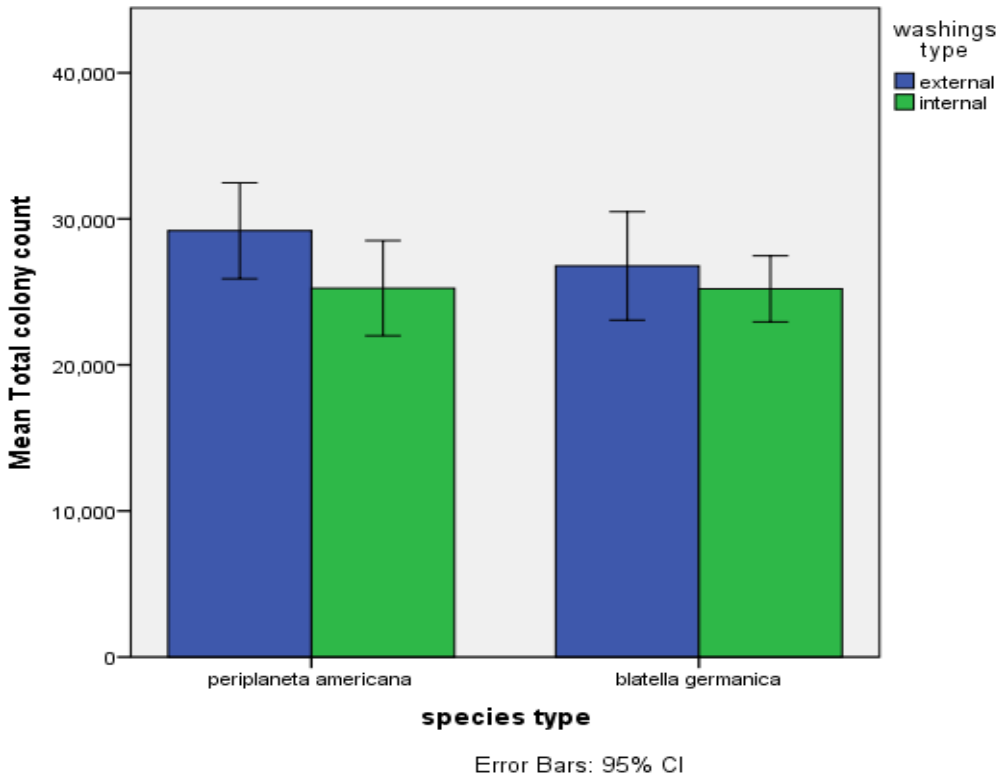


Figure 4.4 Mean Total Bacterial Counts (cfu/ml)



Fighre 4.5 Mean Total Colony count

The mean Total Colony Count for external washings is higher in *Periplaneta* species than in *Blatella germanica* The total colony count for internal washings is almost the same in the internal washings of the two species.

Figure 4.5 Mean Total Coliform Counts (cfu/ml)

(a) (b)

(c)  (d) 

 (e)

Figure 4.6 on top (a) catalase positive test, (b)positive indole test, (c) positive urease test (d)positive citrate utilisation test (e) Kligler iron and lysine test.

4.4ANTIBIOTIC RESISTANCE

Antibiotic resistance patterns were measured using a ruler measuring the diameter of resistance. Resistance pattern were compared to the standard zone size interpretive chart (Appendix 4). There was single to multiple resistance to antibiotics by the isolates. Resistance to amoxicillin, streptomycin and Augmentin was common among the most of the bacterial species; however, other isolates were susceptible to these antibiotics. Gram positives were generally susceptible to chloramphenicol, erythromycin and ciprofloxacin.

CHAP**TER 5 DISCUSSION**

5.1 COCKROACHES AND THE CARRIAGE OF MICROORGANISMS

The purpose of this study was to prove the importance of sanitation in the hospital environment. A greater number of problems can result from insanitary environmental conditions and one of the key problems is the proliferation of such persistent pests such as cockroaches. In Zimbabwe the most commonly found species of cockroaches are the American cockroach(*periplaneta americana)* and the German cockroach (*blatella germanica).Periplanta americana* is approximately 35 to 40 mm in length and *blatella germanica* is 10 to 15 mm. Therefore *Periplanta americana* had a significantly higher bacterial count than *blatella germanica*, this may be due to a larger surface area both on the

external and internal surface of the cockroach (Ahaduzzaman *et al*., 2014). Because *Periplaneta americana* is 3- to 4-fold longer than *B. germanica*, it likely has significantly larger external and internal surface areas. These findings suggest that insect size could correlate with microorganism carriage. In addition to bacteria, in other studies done in Taiwan Salehzadeh *et al* (2007)many species of parasites were isolated from external surface and guts of cockroaches included cysts of *Entameoba coli*, *Entameoba histolytica* and *Giardia lamblia* and adult and ova of *Enterobius vermicularis* and ova of *Hymenolepis nana* and *Ascaris lumbricoides* (Hsueh *et al*., 2002).

Although *Periplaneta* harboured more total bacterial count than *Blatella germanica* it was observed that the type of species of bacteria found in *Blatella germanica* (5 types of species) were more than that of *Periplaneta americana* (3 types of species). This result is due to the roaming territory differences between the two species and they are also known to have different personalities regarding how they seek shelter (Akinjoginla *et al*., 2012). This mean that *Blatella germanica* may be a more important potential vector of health acquired infections. In many other studies, the German cockroach has been reported to harbour a considerable number of pathogenic bacteria and fungi and other symbiotic protozoans which are able to digest cellulose (Heidari *et al.,* 2015).

In this study, the microorganisms which were isolated from external surface of cockroaches were higher from that isolated from the internal surfaces. This demonstrated that bacteria and parasites may be disseminated by contact more than their food habits. Ingestion, intestinal transit of these organisms and their subsequent diffusion by faeces are not an absolute necessity before cockroaches can disseminate organism and become involved in spreading diseases (Hamid and Shahnaz, 2012). Many studies have also revealed the predominance (up to 88 %) of isolates of Gram negative bacteria on the cuticle of cockroaches. Most of them belong to the group Enterobacteriaceae. In fact, Blattaria are considered to be an ecological niche of some Enterobacteriaceae (Russell and Jarvis, 2001).

5.1.1 ISOLATED BACTERIA

In this study five potentially pathogenic bacterial species were carried by cockroaches in great numbers. Although in this study a small number of the pathogenic species have been observed compared to other studies done by Feizhaddad *et al*.,(2012), this was due to inadequate equipment and resources for a large scale study. Areas where people have low level immunity (patients) should be aware of bacteria contamination from cockroaches

(Carter and Cole, 2012). In a study carried out by Vazirianzadeh *et al*. (2008) the isolated bacteria were, *Bacillus cereus, Bacillus subtilis, and S. aureus , E. coli, K. pneumoniae, Neisseri*a species the study was mainly based on the sampling of external body and faeces pellets of Egyptian cockroaches in different regions of Ahvaz city (Southwest Iran). In another study carried out at the medical centres of Khorramshahr County in Iran, pathogenic microorganisms were isolated from the external surfaces of all American cockroaches: *Klebsiella* (47.9%), *Pseudomonas* (37%), *E. coli* (30.1%), *Staphylococcus* (24.6%), *Enterobacter* (19.2%), *Streptococcus* (15.1%), *Serratia* (8.2%), *Bacillus* (4.1%), and *Proteus* (2.7%) (Keseler *et al*., 2009). The study revealed that the place of collecting the cockroaches can affect on results according to transmitted microorganisms: *Klebsiella* and *Enterobacter* were more prevalent among the home cockroaches than in those from hospital environment. Amongst the most common bacterial pathogens encountered in the hospital setting in this study were:

5.1.2 ESCHERICHIA COLI

Escherichia coli is a Gram-negative, rod-shaped, coliform bacterium of the genus Escherichia. The presence of *E.coli* was also confirmed by the biochemical tests that were done. Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts. *Escherichia coli* is a facultative anaerobe which constitute 0.9% of gut flora and their faecal-oral transmission is the major route through which pathogenic strains of the bacterium cause disease (Tenaillan *et al*., 2010). Cells are able to survive outside the body for a limited amount of time, which makes *Escherichia coli* the most important indication in hospital environmental surveillance as a measure of faecal contamination. In this study *Escherichia coli* was isolated in all the tested samples of cockroaches. The presence of *E.coli* could indicate the degree of faecal contamination that is taking place in the two sampling sites and most probably the fact that cockroaches are being exposed to the patient's faecal matter as this is confirmed by the presence of *E. coli*. Cockroaches are able to spread this bacteria through mechanical means during their nocturnal movements. The presence of *E.coli* are also associated with many outbreaks of gastro-enteritis, food poisoning and dysentery (Devrajani *et al*., 2009).

A study which was conducted in Rawalpindi hospital revealed that all collected cockroaches carry *S. aureus* and *E. coli* on their external body surface (Mlso *et al*., 2005). This study also indicated almost the same result. Indoor sanitary condition has been significantly correlated

with infestation of cockroaches. Therefore prevention of such bacteria can only be done through thorough pest control practices in hospitals and in homes (Rajagopala *et al*., 2014).

5.1.3 STAPHYLOCOCCUS AUREUS

Staphylococcus aureus is a Gram positive bacteria and is a facultative anaerobe. In this study,7 samples were positive for *Staphylococcus aureus* and this was confirmed by the Gram staining, catalase positive and coagulase positive. Pathogenic strains produce enterotoxins which, when ingested, can cause gastroenteritis (Lowy, 2012).The presence of *staphylococcus* in both the internal surface of the cockroaches and the internal surface proved that cockroaches are responsible for most *Staphylococcal* infections found in hospital settings (Heymann, 2002).There are many strains of *Staphylococcus aureus* and as such it is easy to spread around a hospital facility causing nosocomial infections (Hennekinne, 2012).

Strains of bacteria that are resistant to beta-lactam antibiotics are called methicillin-resistant *Staphylococcus aureus* (MRSA) (Tang *et al*., 2015). Methicillin resistant *S. aureus* (MRSA) is also one of the supreme causes of nosocomial infections in hospitals. 40% to 70% hospital associated infections are chiefly caused by *S. aureus* in intensive care units (ICUs) in which MRSA is a major cause of HAIs (Lin *et al*., 2012). Health workers and medical staff are more likely to contract MRSA infections. These infections will ultimately lead to severe illness and deaths of medical staff and workers working on pathogenic microorganisms (Mahmood *et al*., 2010) and these in turn would have been spread by the movements of cockroaches.

5.1.4 KLEBSIELLA PNEUMONIA

In this study *Klebsiella pneumonia* was isolated in four samples of both species of cockroaches. The existence of the bacteria was observed by growing the samples on MacConkey in which it produced mucoid pink colonies indicating its lactose fermenting characteristics(Tortora, 2010). *Klebsiella Pneumonia* is a type of Gram-negative bacteria that causes different types of healthcare-associated infections, including pneumonia, bloodstream infections, wound or surgical site infections, and meningitis. The existence of such a bacteria *klebsiella pneumonia* in cockroaches is a hazard to health. It has since been observed by scientists that *Klebsiella* has increasingly developed antimicrobial resistance, most recently to the class of antibiotics known as carbapenems, carbapenem antibiotics often are the last line

of defence against Gram-negative infections that are resistant to other antibiotics(Siegel *et al*., 2007).

5.1.5 PSEUDOMONAS AERUGINOSA

Pseudomonas aeruginosa is a common Gram-negative, rod-shaped bacterium. In this study, 4 samples were positive for this bacteria *P. aeruginosa* (Knockgether *et al*., 2011). Even though cockroaches are not themselves pathogenic they carry such pathogenic bacteria (Horner *et al*., 2012). *Pseudomonas* infections are generally treated with antibiotics. Unfortunately, in hospitalized patients, *Pseudomonas* infections, like those caused by many other hospital bacteria, are becoming more difficult to treat because of increasing antibiotic resistance (Funke, 2016). Due to multi drug resistance pattern in most bacteria isolates, selecting the right antibiotic usually requires that a specimen from a patient be sent to a laboratory to test to see which antibiotics might still be effective for treating the infection (Karlowsky *et al*., 2009).

Multidrug-resistant *Pseudomonas* can be deadly for patients in critical care. An estimated 51,000 healthcare-associated *P. aeruginosa* infections occur in the United States each year. More than 6,000 (13%) of these are multidrug-resistant, with roughly 400 deaths per year attributed to these infections (Leach *et al*., 2016). Multidrug-resistant *Pseudomonas* was given a threat level of serious threat in the CDC AR Threat report (Ryan and Ray, 2004).

5.1.6 PROTEUS SP

Proteus vulgaris was isolated from cockroaches in this study and the striking microbiological characteristic of Proteus species is their swarming activity and fishy odour on MacConkey agar. Swarming appears macroscopically as concentric rings of growth emanating from a single colony or an inoculum (Adler *et al*., 2013; (Armbbruster and Mobley, 2012). P. mirabilis. ,P. vulgaris and P. penneri are easily isolated from individuals in long-term care facilities and hospitals and from patients with underlying diseases or compromised immune systems and causes 90% of Proteus infections (Bauchillan *et al*., 2013)(These bacteria disseminated by cockroaches and other mechanical vectors causes nosocomial infections which results in higher motility and costs Doern *et al*., 2005). There has also been one case study of P. vulgaris causing bacteremia and brain abscesses, with the suspected point of entry being the digestive tract (Chong *et al* ., 2013).

5.2 QUANTITATIVE ANALYSIS

The mean bars show the possible error that could have been done while counting both the total colony count and the total bacterial count. Where the bars does not span the whole bar it shows that those results are significant.

5.3 ANTIBIOTIC RESISTANCE PATTERNS

Infections due to bacteria are treated with antibiotics now, due to these multi-resistant patterns these infections are now harder to treat and this effect has mainly been observed in health care settings (WHO, 2015). According to the results obtained there is a high prevalence distribution of antibiotic resistance among common pathogenic bacteria in hospitals in Gweru. Some of the bacterial isolates are known to be pathogenic (*Proteus spp*) while others are either opportunistic pathogens (*Pseudomonas and Klebsiella*) or play roles in food spoilage (*Pseudomonas and Escherichia coli*,). Species belonging to these groups of bacteria have also been confirmed to be cockroach-borne previously (Siachua *et al.,* 2008). Generally, *Klebsiella spp* strains are characterized typically by presence of chromosomally encoded beta-lactamases (class A), which give them resistance to penicillins, and what is more, many of them have beta-lactamases with a wide range of activity. The latter gives them the resistance to carbenicillin, ampicillin and quinolones), which give them resistance to penicillins, and what is more, many of them have beta-lactamases with a wide range of activity. The latter gives them the resistance to carbenicillin, ampicillin and quinolones. It is also documented that the strains of *Klebsiella pneumoniae* and *E. coli*, (especially resistant - for example those which produce ESBL-extended spectrum beta lactamases), have a high ability to spreading of bacteria in the hospital environment.

According to other studies done by Gulani *et al* (2016) The German cockroach *blatella germanica* is known to harbour a considerable number of pathogenic bacteria, protozoans and fungi. Moreover, drug-resistant *Salmonella, Klebsiella,* and *Pseudomonas aeruginosa* have also been isolated from these species. Although we did not isolate Salmonella in this study, Pseudomonas aeruginosa with various levels of resistance to four antibiotics were isolated from the German cockroaches. E.coli was also resistant to four other antibiotics Augmentin, amoxicillin, streptomycin and gentamicin. Amoxicillin and streptomycin and gentamicin had the highest resistance pattern in most of the isolated bacteria (Kassiri *et al*., 2012).

Most common pathogenic bacteria isolated had a greater intrinsic resistance to antibiotics, however in this study a partial potency of some of the tested drugs like chloramphenicol and

ciprofloxacin was observed. In this study, staphylococcus had a greater resistance in amoxicillin, streptomycin and gentamicin this phenotypic resistance pattern was also observed for most of the gram negative bacteria (Fezhaddad *et al*., 2012). These findings indicate the seriousness of antibiotic resistance of common pathogenic bacteria in Gweru. It has been reported that drug-resistant *K. pneumoniae* carried by cockroaches could have caused an outbreak of nosocomial disease (Heidari *et al*., 2015).

However, Tachbele *et al*., (2006) reported that *S. aureus* was resistant to Kanamycin and Cephalothin in Ethiopia. In addition, resistance was also found against other antibiotics such as Ampicillin, Sulfamethoxazole, Polymyxin B, Carbenicillin, Chloramphenicol, Streptomycin, Tetracycline, Augmentin, Clindamycin, Oxacillin, Erythromycin, Penicillin-G, Vancomycin in Ethiopia (Kinfua and Erkob, 2008).Clearly, high levels of antibiotic-resistant isolates comprising the bacteria found in these cockroaches is undesirable to health (Zurek and Schal, 2004). However, in this study due to inadequate resources the number of bacteria found was very few compared to many other studies done by other authors.

5.4 CONCLUSION

The presence of cockroaches in human dwelling areas is never desirable, and must be taken into consideration. Poor hygiene is probably the most important factor in the spread of nosocomial pathogens. It is apparent that multiple drug-resistant bacteria of medical importance were isolated from cockroaches. Although in this study a small number of the pathogenic species have been observed , the presence of immune depressive people is higher in hospitals these people may be in grave danger due to these cockroaches, it is also a risk factor for human health. Cockroach infestation was documented in more than 50% of the hospitals studied. A considerable number of bacteria and fungi were isolated from nearly all cockroaches collected from these hospitals. Moreover, common bacteria pathogenic to humans isolated from the insects were often found to resist many antibiotics. These findings suggest a possible role for cockroaches in the epidemiology of nosocomial infections.

5.5 RECOMMENDATIONS

Cockroaches can also present a real hazard for human health because they can carry several pathogenic and potentially pathogenic bacteria, including other microorganisms. Pest control regulations should therefore increase in the elimination of cockroaches from sensitive areas,

such as hospitals, this practice is very essential. In food-handling establishments and human dwellings, cockroaches must also be controlled, to maintain acceptable hygiene standards. Therefore, further studies are clearly necessary to be carried out in order to investigate relevant control methods against cockroaches using effective health programs focusing on hygiene measures in medical institutions.

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APPENDICES

Appendix 1

The mean total bacterial count and mean total coliform count of the external surface washings of the *periplaneta americana* and *blatella germanica*

Sample	Total bacterial count (cfu/ml)/average counts	Total coliform count (Cfu/ml)/average counts
EWPI	3.60×10^4	3.40×10^4
EWP2	2.80×10^4	2.40×10^4
EWP3	3.44×10^4	3.00×10^4
EWP4	2.90×10^4	2.60×10^4
EWP5	3.85×10^4	3.00×10^4
EWP6	2.56×10^4	2.50×10^4
EWP7	3.70×10^4	3.45×10^4
EWP8	3.60×10^4	3.00×10^4
EWB1	2.50×10^4	2.05×10^4
EWB2	3.10×10^4	2.55×10^4
EWB3	3.50×10^4	3.13×10^4
EWB4	3.00×10^4	2.50×10^4
EWB5	2.52×10^4	2.12×10^4
EWB6	2.90×10^4	2.60×10^4
EWB7	2.94×10^4	2.25×10^4
EWB8	3.20×10^4	3.00×10^4

KEY. EWP-external washings of periplaneta **EWB-external washings of blatella germanica** **Cfu –coliform forming units**

APPENDIX 2

Internal washings

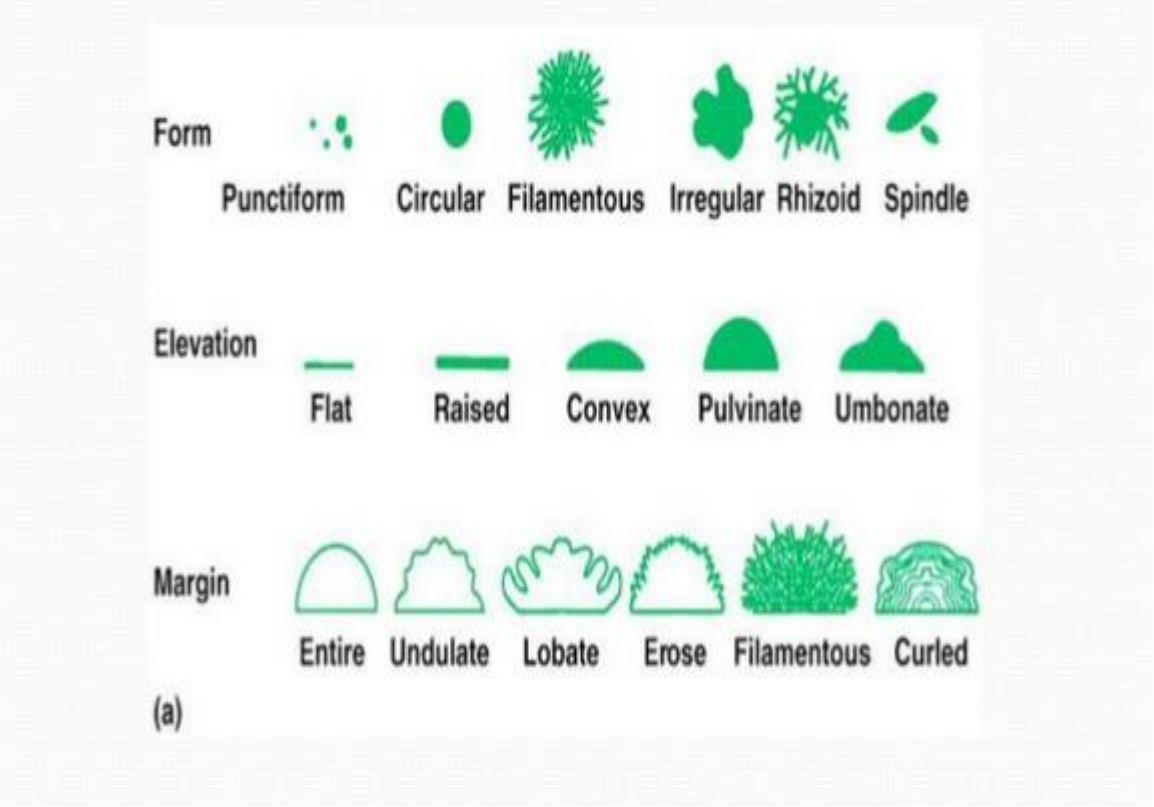
SAMPLE	Mean total bacterial count Cfu/ml	Mean total coliform count Cfu/ml
IWP1	3.40×10^4	3.00×10^4
IWP2	2.40×10^4	2.00×10^4
IWP3	3.00×10^4	3.15×10^4
IWP4	2.60×10^4	2.40×10^4
IWP5	3.00×10^4	2.80×10^4
IWP6	2.50×10^4	2.10×10^4
IWP7	3.45×10^4	2.90×10^4
IWP8	3.00×10^4	3.05×10^4
IWB1	2.05×10^4	2.10×10^4
IWB2	2.55×10^4	2.53×10^4
IWB3	3.13×10^4	2.90×10^4
IWB4	2.50×10^4	2.40×10^4
IWB5	2.12×10^4	2.30×10^4
IWB6	2.60×10^4	2.45×10^4
IWB7	2.25×10^4	2.64×10^4
IWB8	3.00×10^4	2.85×10^4

Key. IWP –Internal washings of periplaneta americana

IWB-Internal washings of blatella germanica

Cfu-coliform forming units, ml-millilitres

APPENDIX 3- MACROSCOPIC IDENTIFICATION(WANI, 2013)



APPENDIX 4

Antibiotic resistance pattern of Gram-negative bacteria isolated from the two samples of cockroaches.

The antibiotic sensitivity tests showed that most of the coliforms displayed resistance patterns to amoxicillin, Augmentin, streptomycin and partly gentamicin. On the other hand almost all *staphylococcus aureus* samples were sensitive to Ciprofloxacin and Erythromycin. Most of the Gram negative bacteria were also sensitive –intermediate to Chloramphenicol, Ciprofloxacin and partly gentamicin.

APPENDIX 4.1 ANTIBIOTIC SUSCEPTIBILITY TESTS

Isolates	**Antibiotics**						**Phenotype of resistance pattern**
	AMX	**STP**	**CHM**	**CPX**	**GEN**	**AUG**	
E.coli							
1	R	R	I	I	S	R	AMX,STP,AUG
2	R	R	S	S	S	I	AMX,STP
3	R	R	S	S	I	R	AMX,STP,AUG
4	R	R	S	S	S	R	AMX,STP,AUG
5	R	R	I	S	R	R	AMX,STP,GEN,AUG
6	R	S	R	R	R	R	AMX,CHM,CPX,GEN, AUG
7	R	R	R	R	S	R	AMX,STP,CHM,CPX, AUG
8	R	S	R	S	I	R	AMX,CHM,AUG
9	I	R	S	I	R	R	STP,GEN,AUG
10	R	S	I	I	R	R	AMX,GEN,AUG
11	R	R	R	S	R	R	AMX,STP,CHM,GEN, AUG
Klebsiella spp							
1	R	S	R	S	R	R	AMX,CHM,GEN,AUG
2	R	S	I	I	R	R	AMX,GEN,AUG
3	R	S	S	S	R	R	AMX,GEN,AUG
4	R	I	S	I	R	I	AMX,GEN
Proteus spp							

1	R	R	I	S	S	R	AMX,STP,AUG
2	R	R	I	S	S	R	AMX,STP,AUG
3	R	I	I	S	S	R	AMX,AUG
4	I	R	R	S	I	R	STP,CHM,AUG
5	R	I	R	S	R	R	AMX,CHM,GEN,AUG
Ps.aeruginoosa							
1	R	R	I	S	S	R	AMX,STP,AUG
2	R	R	R	S	S	R	AMX,STP,CHM,AUG
3	R	S	R	S	I	I	AMX,CHM

Key. AMX- Amoxicillin 30µg, CPX- Ciprofloxacin 10 µg , GEN- Gentamicin 10 µg, STP- Streptomycin 30 µg ,CHM- Chloramphenicol 30 µg , AUG- Augmentin 25 µg,

Appendix 4.2

ANTIBIOTIC RESISTANCE PATTERN OF GRAM POSITIVE BACTERIA

Isolate	**Antibiotics**					**Phenotype of resistance**
Staphylococcus aureus	**AMX**	**STP**	**CPX**	**ERY**	**GEN**	
1	**S**	**I**	**S**	**R**	**R**	**GEN,ERY**
2	**R**	**R**	**S**	**S**	**R**	**AMX,STP,GEN**
3	**I**	**R**	**R**	**S**	**R**	**STP,GEN,CPX**
4	**R**	**R**	**S**	**S**	**R**	**AMX,STP,GEN**
5	**R**	**R**	**S**	**S**	**R**	**AMX,STP,GEN**
6	**R**	**R**	**S**	**I**	**R**	**AMX,STP,GEN**
7	**R**	**R**	**S**	**I**	**R**	**AMX,STP,GEN**

Key. AMX- Amoxicillin 30µg, CPX- Ciprofloxacin 10 µg , GEN- Gentamicin 10 µg, STP- Streptomycin 30 µ ,CHM- Chloramphenicol 30 µg , AUG- Augmentin 25 µg, ERY- Erythromycin 10 µg.

APPENDIX 5
ZONE OF INTERPRETIVE CHART

Antimicrobial agent	Symbol	Disc content	Resistant/mm	Intermediate/mm	Sensitive/mm
Amoxicillin	**Am**	**30**	**13**	**14-16**	**17**
Chloramphenicol	**Chm**	**30**	**12**	**13-17**	**18**
Streptomycin	**Stp**	**10**	**11**	**12-14**	**15**
Gentamicin	**Gen**	**10**	**12**	**13-14**	**15**
Ciprofloxacin	**Cpx**	**10**	**24**	**25-33**	**34**
Augmentin	**Aug**	**25**	**13**	**14-17**	**18**
Erythromycin	**Ery**	**10**	**12**	**13-17**	**18**

APPENDIX 6

6.1-normality

Tests of Normality[a]

	species type	Kolmogorov-Smirnov[b]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
total bacterial count	periplaneta	.235	8	.200*	.885	8	.208

*. This is a lower bound of the true significance.

a. species type = periplaneta, washings type = external washings

Tests of Normality[a]

	species type	Kolmogorov-Smirnov[b]			Shapiro-Wilk		
		Statistic	Df	Sig.	Statistic	df	Sig.
TBC	Periplaneta	.223	8	.200*	.888	8	.224

*. This is a lower bound of the true significance.

a. species type = periplaneta, washings = internal

Tests of Normality[a]

	species type	Kolmogorov-Smirnov[b]			Shapiro-Wilk		
		Statistic	Df	Sig.	Statistic	df	Sig.

TBC	blatella germanica	.181	8	.200*	.947	8	.685

*. This is a lower bound of the true significance.

a. species type = blatella germanica, washings = external

Tests of Normality[a]

	species type	Kolmogorov-Smirnov[b]			Shapiro-Wilk		
		Statistic	Df	Sig.	Statistic	df	Sig.
TBC	blatella germanica	.195	8	.200*	.915	8	.394

*. This is a lower bound of the true significance.

a. species type = blatella germanica, washings = internal

b. Lilliefors Significance Correction

6.2-homogeneity tests

Levene's Test of Equality of Error Variances[a]

Dependent Variable: total bacterial count

F	df1	df2	Sig.
2.341	3	28	.095

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + species + washings + species * washings

6.3 Two -way anova table

Tests of Between-Subjects Effects

Dependent Variable: TBC

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	107342109.375[a]	3	35780703.125	2.502	.080	.211
Intercept	29722267578.125	1	29722267578.125	2078.128	.000	.987
Species	84013203.125	1	84013203.125	5.874	.022	.173
Washings	22865703.125	1	22865703.125	1.599	.217	.054
species * washings	463203.125	1	463203.125	.032	.858	.001
Error	400467812.500	28	14302421.875			
Total	30230077500.000	32				
Corrected Total	507809921.875	31				

a. R Squared = .211 (Adjusted R Squared = .127)

5.4 Graph showing interaction of factors

Appendix 7 -Total colony count

7.1

Tests of Normality[a]

	washings type	Kolmogorov-Smirnov[b]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	Df	Sig.
Total colony count	external	.207	8	.200*	.908	8	.339

*. This is a lower bound of the true significance.

a. washings type = external, species type = periplaneta Americana

Tests of Normality[a]

	washings type	Kolmogorov-Smirnov[b]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	Df	Sig.
Total colony count	external	.238	8	.200*	.878	8	.182

*. This is a lower bound of the true significance.

a. washings type = external, species type = blatella germanica

Tests of Normality[a]

	washings type	Kolmogorov-Smirnov[b]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	Df	Sig.
Total colony count	internal	.174	8	.200*	.932	8	.538

*. This is a lower bound of the true significance.

a. washings type = internal, species type = periplaneta Americana

Tests of Normality[a]

	washings type	Kolmogorov-Smirnov[b]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	Df	Sig.
Total colony count	internal	.138	8	.200*	.970	8	.896

*. This is a lower bound of the true significance.

a. washings type = internal, species type = blatella germanica

7.2 Homogeneity test

Levene's Test of Equality of Error Variances[a]

Dependent Variable: Total colony count

F	df1	df2	Sig.
1.044	3	28	.389

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + species + washings + species * washings

7.3 Two way anova table

Tests of Between-Subjects Effects

Dependent Variable: Total colony count

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	83803359.375[a]	3	27934453.125	1.939	.146	.172
Intercept	22655221953.125	1	22655221953.125	1572.510	.000	.983
Species	11943828.125	1	11943828.125	.829	.370	.029
Washings	60637578.125	1	60637578.125	4.209	.050	.131
species * washings	11221953.125	1	11221953.125	.779	.385	.027
Error	403397187.500	28	14407042.411			
Total	23142422500.000	32				
Corrected Total	487200546.875	31				

a. R Squared = .172 (Adjusted R Squared = .083)